Investigating DHCR24 as a protector against cellular stress: More than just a cholesterol-synthesising enzyme

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Certificate of Original Authorship

This thesis is the result of a research candidature conducted jointly with another University as part of a collaborative Doctoral degree. I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as part of the collaborative doctoral degree and/or fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Date:

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Abstract

Atherosclerosis and insulin resistance are globally prevalent metabolic diseases, primarily driven by endothelial and hepatic inflammation, respectively. High density lipoprotein (HDL) reduces the inflammation in models of atherosclerosis and insulin sensitivity, and in doing so, improves these conditions. Our laboratory has demonstrated that in human coronary artery endothelial cells (HCAECs), HDL's suppression of the inflammatory response is a gene-regulated process and that 3β-hydroxysteroid-Δ24 reductase (DHCR24) is one of the most upregulated genes by HDL.

DHCR24 is a cholesterol biosynthesis enzyme however, recent work in various cell types shows DHCR24 emerging as a potent multifaceted protein protecting against cellular stress. This study commenced with confirming apolipoprotein I (apoA-I) rHDL increases DHCR24 expression in HCAECs, while revealing for the first time that HDL also increases DHCR24 expression in human hepatoma 7 (HuH7) cells.

This study next questioned whether DHCR24 mimics apoA-I rHDL's suppression of the inflammatory response in these cell types. The data presented provides proof-of-principle that DHCR24 replicates HDL's suppression of tumour necrosis factor-alpha (TNF-α)-induced intracellular cell adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) levels in HCAECs, and interleukin 8 (IL-8) levels in HuH7 cells.

Characterising DHCR24's role was explored to learn more about how DHCR24 suppresses a TNF-α-induced inflammatory response in HCAECs and HuH7 cells. This study showed that DHCR24's oxidoreductase region mediates DHCR24's suppression of a TNF-α-induced inflammatory response in these cell types. This effect occurred without DHCR24 increasing cholesterol levels, despite the oxidoreductase site's integral role in cholesterol biosynthesis. These are exciting results as they indicate that DHCR24 is more than just a cholesterol biosynthesis enzyme in HCAECs and HuH7 cells.

Elucidating the mechanisms by which DHCR24 suppresses a TNF-α-induced inflammatory response in HCAECs and HuH7 cells highlighted a cell type-specific nature of DHCR24's activity, which is in keeping with reports in the literature. In keeping with this cell type-specificity, a TNF-α-induced inflammatory response was suppressed, in part, by a decreased endoplasmic reticulum (ER) stress response in HCAECs. Interestingly, this did not occur in HuH7 cells. The work here suggests that this is attributed to the increased cholesterol content in HuH7 cells

compared to HCAECs. DHCR24's cell type-specific effects are reinforced by the cellular response of DHCR24 levels to TNF-α-activation – in HCAECs, DHCR24 levels were modestly increased; conversely in HuH7 cells, TNF-α-activation markedly decreased DHCR24 levels. Moreover, TNF-α-activation caused DHCR24's translocation from its ER-localisation to the cytoplasm and nucleus, while in HuH7 cells, DHCR24 remained localised peri-nuclearly.

This data provides novel mechanistic insight into DHCR24's multifunctional role in two different cell types, laying the foundation for potential DHCR24-based therapeutics, and provides impetus for further investigation of DHCR24.

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List of Abbreviations

3’	Three prime
5’	Five prime
18S	18*S* ribosomal *RNA*
28S	28S ribosomal RNA
w/v	Weight per volume
Aβ	Amyloid beta
ABCA1	ATP binding cassette transporter A1
ABCG1	ATP binding cassette transporter G1
ABS	Australian bureau of statistics
ABTS	2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt
ACC1	Acetyl-CoA carboxylase 1
ACC2	Acetyl-CoA carboxylase 2
ACTH	Adrenocorticotrophic hormone
AIHS	Australian Institute of Health and Welfare
ANOVA	Analysis of variance
ApoA-I	Apolipoprotein I
ApoE	Apolipoprotein E
$ApoE^{-/-}$	Apolipoprotein E homozygous knockout
ATF-4	Activating transcription factor-4
ATF-4(n)	Active nuclear form of ATF-4
ATF-6	Activating transcription factor-6
ATP	Adenosine triphosphate
ASK1	Apoptosis signal-regulating kinase 1
β2M	Beta-2 Microglobulin
BCA	Bicinchoninic acid assay
bFGF	Basic fibroblast growth factor
BSA	Bovine serum albumin
C-terminal	Carboxyl-terminal
CAM	Cell adhesion molecule
CCL2	Chemokine ligand 2
CCL5	Chemokine ligand 5
CD40L	cluster of differentiation 40L
cDNA	Complementary deoxyribonucleic acid
CETP	cholesteryl ester transfer protein
CHOP	CCAAT enhancer binding protein homologous protein
$Chop^{-/-}$	Chop homozygous knockout
CO_2	Carbon dioxide
CRP	C-reactive protein
COX	Cyclooxygenase
COX1	Cyclooxygenase 1
COX 2	Cyclooxygenase 2
CX_3CR1	CX3C chemokine receptor 1
DAB	3,3’-diaminobenzidine
DAPI	4',6-diamidino-2-phenylindole
DHCR24	3β-hydroxysteroid-Δ24 reductase
DMEM	Dulbecco’s Modified Eagle Medium

DNA	Deoxyribonucleic acid
E.coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
EGFP	Enhanced green fluorescent protein
ELISA	Enzyme linked immunosorbent assay
EIF2α	Eukaryotic initiation factor 2 alpha
eNOS	Endothelial NO synthase
ER	Endoplasmic reticulum
ERO1α	ER oxidase 1 alpha
FAD	Flavin adenine dinucleotide
FBS	Fetal bovine serum
FDA	Food and Drug Administration
FFA	Free Fatty Acid
FBG	Fasting blood glucose
FITC	Fluoroscein-Isothiocyanate
FPLC	Fast protein liquid chromatography
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GIT	Gastrointestinal tract
GM-CSF	Granulocyte-macrophage colony stimulating factors
GRP78	Glucose-regulated protein 78
H&E	Haematoxylin and eosin
H_2O	Water
H_2O_2	Hydrogen peroxide
HbA1c	Glycated haemoglobin
HBSS	Hank's buffered salts solution
HCAECs	Human coronary artery endothelial cells
HCV	Hepatitis C virus
HDL	High-density lipoprotein
HFD	High fat diet
HO-1	Heme oxygenase-1
HRP	Horseradish peroxidase
HS	Human serum
hs-CRP	High sensitivity C-reactive protein
HuH7	Human hepatoma 7
HUVEC	Human umbilical vein endothelial cells
ICAM-1	Intracellular cell adhesion molecule 1
IFN-γ	Interferon-gamma
IGF-1	Insulin-like growth factor-1
IKK	IκB kinase
IL-1	Interleukin-1
IL-1β	interleukin 1-beta
IL-6	Interleukin-6
IL-8	Interleukin-8
ILLUMINATE	Investigation of Lipid Level Management to Understand Its Impact in Atherosclerotic Events
IRE1	Inositol 1,2,5-triphosphate-activated receptor
JNK	c-Jun amino-terminal kinase
KBr	Potassium bromide
LB	Luria broth
LDL	Low-density lipoprotein

$Ldlr^{-/-}$	LDL receptor homozygous knockout
M-CSF	Monocyte colony stimulating factor
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemoattractant protein-1
MEF	Mouse embryonic fibroblast
MHC	Major histocompatibility complex
MMP	Metalloproteinase
N-terminal	Amino-terminal
NaCl	Sodium chloride
NADPH	Nicotinamide adenine dinucleotide phosphate
NCBI	National Center for Biotechnology Information
NCD	Non-communicable disease
NFκB	Nuclear factor kappa B
NO	Nitric oxide
NPG	*n*-propyl gallate
OD	Optical density
OGTT	Oral glucose tolerance test
oxLDL	Oxidised low density lipoprotein
p38 MAPK	p38 mitogen-activated protein kinase
p53	Tumour suppressor p53/Protein 53/Tumour protein 53
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PC12	Pheochromocytoma
PCR	Polymerase chain reaction
PDGF	Platelet-derived growth factor
PERK	Protein kinase-like endoplasmic reticulum kinase
PI3K	Phosphatidylinositol 3-Kinase
PLPC	1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphatidylcholine
PVDF	Polyvinylidene fluoride
qPCR	Real-time PCR
RCT	Reverse cholesterol transport
rHDL	Reconstituted high-density lipoproteins
RIPA	Radioimmunoprecipitation assay buffer
RNA	Ribonucleic Acid
ROS	Reactive oxygen species
RPM	Revolutions per minute
RPMI	Roswell Park Memorial Institute
RT-qPCR	Reverse transcription real time PCR
S1P	Site-1 protease
SAA	Serum amyloid A1
SAPK	Stress activated protein kinase
SDS	Sodium dodecyl sulfate
SEM	Standard error of the mean
SH-SY5Y	Neuroblastoma cell line
siRNA	Small interfering ribonucleic acid
SMC	Smooth muscle cell
SR-B1	Scavenger receptor class B type 1
STAT1	Signal transducer and activator of transcription-1
sXBP-1	Spliced X-box binding protein-1
TBS	Tris buffered saline

TGF-β	Transforming growth factor-beta
TNF-α	Tumour necrosis factor-alpha
TRAF2	Tumour necrosis factor receptor-associated factor 2
UPR	Unfolded Protein Response
UV	Ultraviolet
VCAM-1	Vascular cell adhesion molecule-1
WHO	World Health Organisation
XBP-1	X-box binding protein-1